HEMOGLOBIN IN THYONE BRIAREUS LESUEUR.[1]

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Hemoglobin is by no means rare in lower animals. Though it was originally thought to be characteristic for the vertebrates, it has been found in many other instances. Even in animals as low in the scale of phylogenesis as the worms, we find it in very many forms either in the cœlomic liquid, as in the common earthworm, or in corpuscles (*Glycera, Capitella, Phoronis,* etc.). In Molluscs and Arthropods it is generally found in the free form. It is chiefly found in mud-dwellers, or more in general, in animals living in a medium in which oxygen is scarce. The larva of *Chironomus* is a classical example.

Our knowledge of the occurrence of respiratory pigments in Echinoderms is very incomplete. In one case, in the brittle star, *Ophiactis virens,* hemoglobin has been found. Other statements, *e.g.,* the assumption of a respiratory function of the so-called "echinochrome (Mac Munn. 1885.)" in *Echinus* and *Strongylocentrotus,* are to be accepted with some scepticism. Winterstein, *e.g.,* could show that a solution of echinochrome did not take up any more oxygen than the same amount of sea water. Other data concerning respiratory pigments in Echinoderms have not come to the writer's knowledge.

In studying the digestion of the Echinoderms, among which *Thyone briareus,* a dendrochirote Holothurian common around Woods Hole, the writer was struck by the brilliant red color of the Polian vesicles every time he dissected a specimen. Curiosity drove him to examine this color a little more closely. The color appeared to be due to the contents; it "flows away," when the vesicle is cut. It could be centrifuged off and a microscopical

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examination of the fluid showed that it was bound to corpuscles.

These corpuscles are, as far as their appearance under the microscope is concerned, very much like mammalian blood corpuscles; they are however not biconcave discs, but seem to be perfectly round. Their color is the same as that of mammalian corpuscles. They do not have any active movements as far as could be observed, and under the microscope they have the appearance of perfectly round discs. A nucleus is clearly visible in most cases; the protoplasm has a granular structure and a cell membrane gives a perfectly clear "outline."

The question arose as to what the function of these corpuscles was. The Polian vesicle forms a part of the water vascular system. In this way they stand on one hand in connection with the ambulacral feet, which are found all over the outside of the body and by means of which the animal moves. This species is radially symmetrical and does not have the so-called bivium. Inside the muscular body wall these podia are represented by their ampullæ and, remarkably enough, all these little ampullæ can be seen on dissection to contain the same colored material. On the other hand, the Polian vesicle, through the water vascular system, communicates freely with the cœlomic fluid. The stone-canal stiffened by carbonate of lime, which in other Echinoderms and in larval Holothurians communicates with the sea water, forms here so-called "internal madreporites," one or even five, as in *Holothuria tubulosa*. This stone-canal is derived from the larval axial sinus, whereas the original pore-canal and external madreporite disappear. Furthermore the water vascular system stands in connection with the tentacles, the "feelers," which by their active movements may assist greatly in respiration.

The so-called blood system of the Holothurians consists of a blood ring surrounding the œsophagus and situated inside of the water vascular ring. This system forms a plexus around the gut and water lungs, a dorsal "vessel," and probably stands in connection with the cœlomic fluid.

The water lungs are considered to be organs of respiration. A rhythmical contraction of the cloacal wall can be seen, resulting in in- and outflow of water into the surrounding sea water.

The question arose as to what the nature of the red pigment in the corpuscles of the Polian vesicle was.

The following evidence will serve to show that true hemoglobin is present in these corpuscles:

1. A small quantity of the fluid was dried on a slide, heated a few times under cover-glass with acetic acid till bubbles appeared, then cooled. Blue-black prismatic crystals could be seen, which though not absolutely congruent with the figures of text-books of biochemistry, doubtlessly were hemin crystals.

2. Centrifuged and "laked" "blood" showed the typical absorption spectrum of oxyhemoglobin—one broad band in concentrated solution, two on dilution, one of which can be easily identified by means of the D—sodium—line.

3. On reduction with Stokes's reagent the single band of hemoglobin could be seen. After continued vigorous shaking, the double band of oxyhemoglobin could again be produced.

4. Material treated with KCN and kept in the thirty-seven-degree incubator for some time, became orange-yellow and showed the absorption band in the green which characterises cyanhemoglobin.

5. A small quantity of the material gave a positive Prussian blue test for iron.

6. To a solution of one or two drops of 1 per cent. benzidine in a large quantity of hydrogen peroxide a few drops of the "blood" were added. A dark blue color developed at once and a vigorous foaming of oxygen could be seen (peroxidase action).

From this evidence the writer concluded that hemoglobin is present in these corpuscles. This is very interesting in connection with the fact that *Thyone* is a mud-dweller, living in a medium in which oxygen is scarce. Though the controversy between Krogh and other investigators, *e.g.,* Jordan, as to whether hemoglobin actually functions as an oxygen *store,* has not yet been settled, it is beyond doubt, that the presence of a respiratory pigment is a great help in the struggle for oxygen.

In how far the Polian vesicle acts as "mover" of the corpuscles, as a kind of primitive heart, could not be investigated more closely. It is strongly contractile however and is generally

supposed to control the hydraulic pressure in the water vascular system. No rhythmical contraction can be observed.

The same corpuscles can be seen in a preparation of the above-mentioned water lungs. This makes their respiratory function still more probable. On the other hand the evidence seems to show that also the podia and the tentacles play an important rôle in the respiration of *Thyone,* and that two systems of organs seem to be concerned with one function.

SUMMARY.

Hemoglobin is found, bound to corpuscles, in the Polian vesicle(s), the ampullæ and the wall of the water lungs of *Thyone briareus,* a dendrochirote Holothurian, common around Woods Hole. This fact is interesting in connection with the fact that *Thyone* is a mud-dweller.